WS23.1 Models of the 3D structure of CFTR: from the understanding of the protein functions to the design of correctors

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In the absence of experimental 3D structures at atomic resolution for the entire CFTR protein, homology models were built from 3D experimental structures of ABC exporters. These models provide valuable insights into the structural and functional characteristics of CFTR. These initial models were recently enriched by other modeling studies and molecular dynamics, offering a description of the possible architecture of the anion channel as a "bottleneck" with a significant narrowing of the pore.
We performed molecular dynamics experiments from our model of the open form of CFTR protein made by homology on the Sav1866 template. This model was validated using structural information provided by new experimental 3D structures of ABC exporters, and by taking into account the internal symmetry involving the two halves of the protein. We were able to explore the stability and the conformational variability of the 3D structure. We obtained a relevant model of the full open form of the anion channel, particularly well consistent with the experimental data available today. A lateral access path for ions and molecules from the cytosol was also highlighted.
These models provide insight into the molecular mechanisms ensuring the proper functioning of the CFTR protein, as well as the impact of mutations in patients with cystic fibrosis (CFTR2 database). In addition, we also use models of mutated forms of CFTR (F508del-CFTR in particular) to perform rational drug design. A series of molecules with a potential corrective effect are currently synthesized and their biological activity evaluated by functional tests.
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WS23.2 Characterization of the CFTR mutation c.3700 A>G informs strategies for future medical intervention

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The *CFTR* variant c.3700 A>G, predicted to cause the missense mutation p.Ile1234Val or alternative splicing, exhibits variable CF disease severity [1]. This variant, while rare in North America and Europe, is relatively common in the Middle East (~12% of CF population). The purpose of this study was to determine the consequences of this mutation. Clinical assays revealed that individuals homozygous for c.3700 A>G exhibited defective CFTR function. Genomic DNA from these patients was sequenced, and total RNA extracted from epithelial cells was transcribed into cDNA and sequenced. We found that this mutation in exon 19 activates a cryptic donor splice-site 18 bp upstream of the original donor splice-site, resulting in the in-frame deletion of 6 amino acids (r.3700_3717del; p.Ile1234_Arg1239del). A CFTR cDNA clone was constructed containing the deletion (p.Ile1234_Arg1239del) and heterologously expressed to test CFTR protein biosynthesis. This deletion, like the major CF-causing mutation p.Phe508del, caused a primary defect in processing. Importantly, Lumacaftor (VX-809), currently in clinical trial for CF patients with p.Phe508del, partially ameliorated the processing defect exhibited by p.Ile1234_Arg1239del. These studies highlight the need to define molecular and clinical consequences of rare *CFTR* variants in order to define possible therapeutic strategies.
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Reference(s)

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WS23.3 ICM is sensitive to detect potentiation of CFTR-mediated Cl^- secretion in patients with cystic fibrosis and the G551D mutation treated with ivacaftor

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Objectives: Sensitive outcome measures of CFTR function may facilitate the implementation of mutation-specific therapy with CFTR modulators in patients with cystic fibrosis with non-G551D mutations. Intestinal current measurement (ICM) is a sensitive assay for functional assessment of mutant CFTR in rectal biopsies and was recently shown to detect potentiator effects of 1-EBIO *ex vivo* (Roth E. et al., PLOS One 2011). The aim of this study was to determine, if ICM is sensitive to detect potentiation of CFTR-mediated Cl^- secretion in rectal epithelia from CF patients with a G551D mutation treated with ivacaftor.
Methods: Rectal biopsies were obtained from 8 patients carrying a G551D-CFTR mutation before and at least four weeks after the start of ivacaftor therapy. Rectal tissues were mounted in micro-Ussing chambers and CFTR-mediated Cl^- secretion was determined from Cl^- secretory responses induced by cAMP (IBMX/forskolin)- and Ca^{2+} (charbachol)-mediated stimulation.
Results: Before ivacaftor therapy, ICM detected variable residual CFTR-mediated Cl^- secretion in rectal tissues from CF patients with a G551D mutation. In the presence of ivacaftor therapy, CFTR-mediated Cl^- secretory responses were increased in all 8 patients.
Conclusion: We conclude that ICM is sensitive to detect in vivo potentiation of mutant CFTR function by treatment with ivacaftor. Our results indicate that ICM may be a useful bioassay to determine therapeutic responses at the level of the basic CF defect of ivacaftor and potentially other clinical CFTR modulators in CF patients with non-G551D mutations.

WS23.4 Rare CF genotype with severe hepatic failure associated with medium chain acid deficiency (MCAD) in a neonate

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We report the case of young baby boy born in Romania, admitted to our emergency department at the age of 1 month for severe neonatal cholestasis associated with coagulation dysfunction and pulmonary infection with multiple pathogens. The first hypothesis was a immune deficiency but not confirmed. The Guthrie test performed at that time was normal for the CF screening, but evidenced a MCAD, which was subsequently confirmed by the presence of the mutation C985A>G. The appropriate food regimen was started. Because of continued clinical deterioration and of lack of diagnosis despite broad etiological research, a sweat test was performed at 2 months of age, pathological. The screening of the CFTR gene confirmed CF diagnosis and found a rare homozygous mutation c.1853_1863del. Fecal elastase was low. Despite aggressive medical and surgical management, he developed severe portal hypertension and ascitis. A liver transplant is considered. Nevertheless, he developed a recurrent right chylothorax, trachebronchomalacia with life-threatening obstructions, and pulmonary infection with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Unfortunately, this child died at the age of 7 months.
Conclusion: This case is of interest for several reasons: (a) This genotype was never described before, (b) and is associated here with very early and severe hepatic dysfunction, which is unusual in CF, (3) the association of CF and MCAD was never reported.